CHAPTER 2

REVIEW LITERATURE

2.1 Longan

The longan (*Dimocarpus longan* Lour. or *Euphoria longana*), belongs to the family Sapindaceae and is a subtropical evergreen fruit tree native to northeastern India, Burma and southern China. It is closely related to the lychee and rambutan. In Thailand, longan production occurs mainly in the northern provinces of Lamphun, Chiang Mai, and Chiang Rai. The seasonal harvest is from late June to late August. However, the fruit is available year-round with off-season production due to the application of potassium chlorate ($KClO_3$), a flower-inducing substance (Qiu *et al*., 2001; Subhadrabandhu and Yapwattanaphun, 2000).

The longan tree usually flowers at the end of winter from December to February depending on the cultivar and climate. The main harvest period is 140-190 days after flowering (Menzel, 1989; Chattopadhyay and Ghosh, 1991; Winston *et al*., 1993; Huang, 1995). Longan fruit, which are borne on panicles of up to 40 fruits, has a sigmoidal growth curve. Initially, the seed and the pericarp develop simultaneously, followed by aril growth (Huang, 1995). Longan fruit are non-climacteric with little change in soluble solids concentration (SSC) or titratable acidity (TA) after harvest. Soluble solids content ranges from 18 to 22% and TA is low with a pH of 6.2 to 6.7. Maturity can be determined on the basis of fruit weight, skin colour, flesh sugar concentration, flesh acid concentration, sugar~~-acid~~:acid ratio, flavour and/or days from anthesis. ~~-~~In practice, harvest maturity is usually assessed on the basis of fruit colour and flavor (Shi, 1990).

~~Thailand is the world's largest producer and exporter of longan. In 2008, Thailand produced approximately 476,930 tons of longan, (Office of Agricultural Economics, 2009). Approximately 32% of the crop was exported as fresh longan in 2007, earning about US $61 million. About 70% of the total crop is exported as fresh,~~

ที่จัดรูปแบบ: ระยะห่างบรรทัด: สองเท่า

ที่จัดรูปแบบ: ระยะห่างของหัวกระดาษจากขอบ: 1", ส่วนหัวของหน้าแรกต่างกัน

ที่จัดรูปแบบ: การจัดเต็มแนวที่กระจายแบบไทย

ที่จัดรูปแบบ: การจัดเต็มแนวที่กระจายแบบไทย, ไม่ปรับช่องว่างระหว่างข้อความละติน และข้อความเอเชีย

ที่จัดรูปแบบ: การจัดเต็มแนวที่กระจายแบบไทย

dried, or canned longan, while the remaining 30% is sold in the Thai domestic market as fresh fruit (Anupunt and Sukhyibul, 2005). The main commercial cultivar is cv.

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ที่จัดรูปแบบ: การจัดเต็มแนวที่กระจายแบบไทย, การเยื้อง: บรรทัดแรก: 0.5"

ที่จัดรูปแบบ: ส่วนหัวของหน้าแรกต่างกัน

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ที่จัดรูปแบบ: การเยื้อง: บรรทัดแรก: 0"
ที่จัดรูปแบบ: ระยะห่างของหัวกระดาษจากขอบ: 1"

ที่จัดรูปแบบ: การจัดเต็มแนวที่กระจายแบบไทย, การเยื้อง: บรรทัดแรก: 0.5"
ที่จัดรูปแบบ: การจัดเต็มแนวที่กระจายแบบไทย

2.2 ~~Fruit l~~Longan fruit morphology and anatomy

The mature longan fruit is small, with a~~;~~ diameter range~~s~~ of ~~from~~ 1 to 3 cm, a conical, heart~~-shaped~~ or spherical ~~in~~ shape, and a light brown ~~in~~ color. Longan fruit consists of a leathery pericarp, an edible translucent white ~~–~~colored aril and a single centrally-located, dark brown seed. Mature fruit weight varies from 5 to 20 g across cultivars (Huang, 1995).

Longan pericarp consists of three layers, an~~;~~ exocarp, mesocarp and endocarp. The exocarp has a thin discontinuous cuticle with many natural openings and cracks, trichomes, a uniseriate epidermis and subepidermal sclerenchyma. The mesocarp, mostly part of the pericarp, consists of parenchymatous cells, large intercellular spaces, vascular tissue and some stone cells. Mitochondria and cell organelles are found within the cells in the mesocarp. The endocarp is made up of small, thin-walled unsuberized epidermal cells and is detached from the aril when mature. The aril of longan is composed of parenchymatous tissue, large vacuoles and small intercellular spaces (Jiang *et al*., 2002; Jaitong, 2006; Suwanakood, 2007).

2.3 Fruit decay of longan

Fruit~~s~~ decay occurs due to two factors: abiotic and biotic. The abiotic factors include various physiological changes ~~induced~~ occurring after harvest and during transit and storage. Hydrolytic action of enzymes, oxidation of fats and metabolism of proteins are the important physiological activities initiated after harvest leading to various degrees of decay. The biotic factors are the microbial action associated with fungi and bacteria. ~~–~~Longan fruit deteriorates rapidly unless proper handling techniques are employed. The two major factors reducing the storage life and marketability of longan fruit are microbial decay and pericarp browning. Low temperature storage at 1–5°C is used to reduce pathological decay, but it has ~~an~~ only a limited effect in reducing pericarp browning (Reed, 1986; Paull and Chen, 1987).

Longan fruit has very high sugar content and low acids in the aril that makes them susceptible to microbial decay. Fungi reported as causes of postharvest disease in longan include *Lasiodiplodia* sp., *Phomopsis* sp., *Pestalotiopsis* sp., *Cladosporium* sp., *Penicillium* sp., *Fusarium* sp., *Curvu~~ral~~aria* sp., *Colletotrichum* sp., and *Rhizopus* sp. etc. (Nachaiwieng, 1994; Rasrinaul, 1996; Withee, 1997; Rimpranam and Sangchoed, 2002). Initial fungal infection often occurs in the field which is latent. Nachawieng (1994) reported the fungal species that were evident during both pre- and postharvest ~~were~~ included *A. flavus, A. niger, L. theobromae, Colletotrichum* sp., *Fusarium* sp., *Pestalotiopsis* sp. However, fruit decay ~~in~~ of longan ~~exocarp~~ pericarp may be caused by fungal spores and mycelia, yeast, and bacteria. Some of them are able to cause necrosis and rot the fruit.

Lasiodiplodia theobromae 'LP 20' is the most virulent fungal isolate which rapidly causes necrosis and rot of longan (Suwanakood, *et al*., 2004). ~~–~~After inoculation with *L. theobromae~~asiodiplodia sp.~~* 'LP20', the inoculation point darkened within 6 h. Mycelia spread rapidly over the pericarp tissue both inter- and intracellularly. It penetrated three layers of pericarp within 12 h, softening the peel and aril tissue and rotting the whole fruit within 24 h after inoculation. Fruit inoculated near the stem-end rotted faster than those inoculated at other ~~parts of fruit~~points (Suwanakood, *et al*., 2004).

2.4 *Lasiodiplodia theobromae* description

ที่จัดรูปแบบ: แบบอักษร: ตัวเอียง

Lasiodiplodia theobromae or *Botryodiplodia theobromae* is classified as follows (Punithalingam and Holiday, 1973):

Kingdom: Fungi

Division: Eumycota

Subdivision: Deuteromycotina

Class: Coelomycetes (imperfect fungi)

Order: Sphaeropsidales

Family: Sphaeropsidaceae

Genus: *Botryodiplodia*

Lasiodiplodia theobromae ~~sp.~~ has a large host range and causes serious losses in papaya, mango, lychee, banana, rambutan, mangosteen, durian, coconut, avocado,

jackfruit, custard apple, etc. (Nagarja *et al*., 1971; Srivastava and Tandon, 1971; Sangchote, 1987; Chana *et al*., 1991; Sangchote and Pongpisutha, 1995; Ploetz *et al*., 2003). It requires free moisture on the surface of fruits to germinate and penetrate. Natural openings such as lenticels or stomata and wounds are the primary infection sites for the fungus. Moreover, it can penetrate the unwounded fruit directly. Symptoms of decay include softening of the tissues, color changes of the infected tissue to brown, gum-like substance secretion and foul odor.

Colonies of *L. asiodiplodia theobromae* sp. are initially white, soon becoming black and fast-spreading with immersed and superficial, branched, septate myceliaum. Shiny black pycnidia are produced on the surface. Conidia are initially hyaline, unicellular, subovoid to ellipsoidal, with a granular content. Mature conidia are two-celled, cinnamon to dark brown, thick walled, ellipsoidal, at 20-37 X by 10-17 µm. This fungus can produce large amounts of cell wall lytic enzymes such as pectinase, cellulase and protease (Nagaraja *et al*., 1971). Softening of the peel and aril is associated with the activity of these pectic enzymes secreted by the pathogen during pathogenesis (Barkai-Golan, 2001).

2.5 Structure and composition of the plant cell wall

The plant cell walls consist of 3 layers, middle lamella, primary cell wall and secondary cell wall, with a cuticle generally covering the outer wall of epidermis. Middle lamella is the first layer formed during cell division. It makes up the outer wall of the cell and is shared by adjacent cells. It is composed of pectic compounds and protein. Primary wall is formed after the middle lamella and consists of a rigid skeleton of cellulose microfibrils embedded in a gel-like matrix composed of pectic compounds, hemicellulose, and glycoproteins (Figure 2.1). Secondary wall forms after cell enlargement is completed. The secondary wall is extremely rigid and provides compression strength. It is made of cellulose, hemicellulose and lignin.

Cuticle: The cuticle is composed of cutin, an amorphous substance consisting of hydroxylated and epoxylated fatty acid which are linked to each other by means of ester bonds. The monomers which makes up this polyester are based on fatty acids with either 16 or 18 carbon atoms.

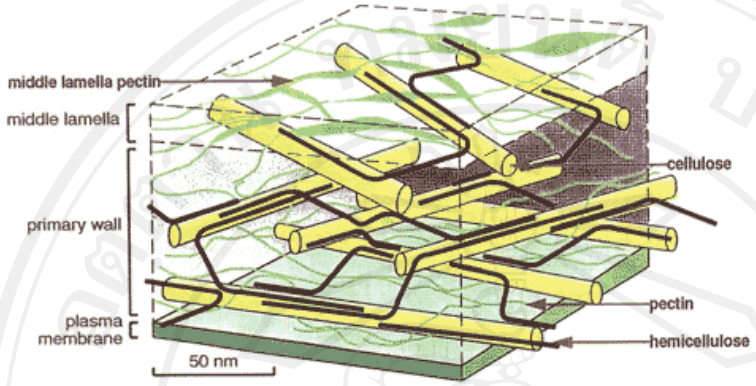

Figure2.1 Plant cell wall

Source: www.daviddarling.info/images/plant_cell_wall.gif on 25 January 2009

~~**Cellulose:**~~ The primary structure of cellulose is an unbranched (1~~,~~4, 4)-linked β-D-glucan. Many parallel glucans snap into register to form a crystalline microfibril that is mechanically strong and highly resistant to enzymatic attack, an almost ideal scaffold material. These long, crystalline ribbons are 3–5 nm wide and, in growing cells, are aligned with each other, giving a structural bias to the cell wall

~~**Hemicelluloses:**~~ The backbone of hemicelluloses resembles that of cellulose. Hemicelluloses bind to cellulose, but branches and other modifications in their structure prevent them from forming microfibrils by themselves. Xyloglucan and arabinoxylan are two of the most abundant hemicelluloses. Details of their structure vary slightly among plant species. Xyloglucan has a backbone that is similar to that of cellulose, but it is decorated with xylose branches on 3 out of 4 glucose residues. The xylose can also be serially appended with galactose (Gal) and fucose (Fuc) residues. Arabinoxylan consists of a (1, 4)-linked β-D ~~d~~-xylan backbone decorated with arabinose branches. Other residues, such as glucuronic acid and ferulic acid esters ~~(FAE)~~, are also attached in arabinoxylans that are particularly abundant in cereal grasses. Mannans are also found in primary cell walls and probably function in the same way as xyloglucan and arabinoxylan.

Pectins: This complex and heterogeneous group of polysaccharides consists of distinctive domains, which are believed to be covalently linked together. Rhamnogalacturan I consists of alternating residues of galacturonic acid and rhamnose, and probably has side branches that contain other pectin domains. Homogalacturonan comprises a linear chain of galacturonic acid residues, whereas xylogalacturonan is modified by the addition of xylose branches. The carboxyl groups of homogalacturonan and xylogalacturonan are often methyl esterified, a modification that 'blocks' the acidic group and reduces their ability to form gels. Rhamnogalacturonan is a complex pectin domain that contains different sugar residues and forms dimers through borate (B) esters. The neutral arabinans and arabinogalactans are also linked to the acidic pectins and it has been proposed that they promote wall flexibility and that they bind to the surface of cellulose.

Lignin: Lignin It is a heterogeneous polymer formed by the polymerization of up to three components,; coumaryl, coniferyl and sinapyl acohol.

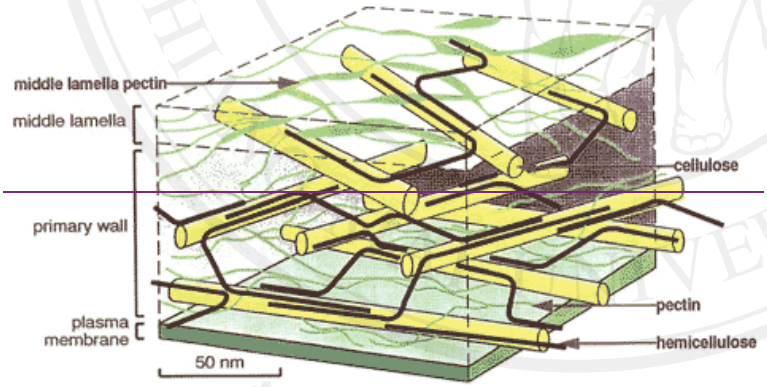

Figure2.1 Plant cell wall
Source: www.daviddarling.info/images/plant_cell_wall.gif availalble on 25 January 2009

2.6 Cell wall degrading enzymes

The plant cell wall is the first barrier to fungal penetration, and penetration appears to be the first requirement for pathogenesis of fungal pathogens. In general,

ที่จัดรูปแบบ: การจัดเต็มแนวที่กระจายแบบไทย, ช่องว่าง ก่อน: 6 พ.

ที่จัดรูปแบบ: การจัดเต็มแนวที่กระจายแบบไทย

plant cell walls are composed of cellulose, hemicellulose, pectic ~~polysaaccharide~~polysaccharides, protein, lignin and incrusting substances such as cuticle, suberin, and certain inorganic compound. Most pathogens much breach both cuticle and cell wall if infection is to occur. Penetration of intact surface may occur by physical processes or by chemical processes or a combination of both. Direct penetration often involves the information of appresoria. However some fungi can also penetrate without forming appresoria. Furthermore, fungal penetration of the host is facilitated by the production of cutinase, followed by softening or disintegration of host tissues by cellulytic and pectolytic enzymes (Agrios, 1988).

Since plant cell walls contain different polysaccharides, proteins and lipids that are interwoven, a single enzyme may not be able to degrade cell walls efficiently. A mixture of different enzymes may be necessary. Many plant pathogenic fungi are known to produce a range of cell wall degrading enzymes that macerate plant cell walls, including cutinase, cellulase, laccase and pectolytic enzymes such as pectin methylesterase (PME), polygalacturonase (PG) and pectin and pectate lyase (PNL and PL, respectively). They may have important roles in the infection process and in the development of disease symptoms (Bateman and Basham, 1976; Walton, 1990). Pectolytic enzymes are often produced in culture and during plant infection sequentially as multiple isoenzymes and may constitute a catabolic pathway for the complete degradation of pectic polysaccharides, the process being initiated by constitutive forms (Leone and Van Den Heuvel, 1987; Chilosi and Magro, 1997). The importance of such enzymes in pathogenicity is supported by the ability of purified enzymes to reproduce disease symptoms (Marciano *et al*., 1982; Barash *et al*., 1984; Holz and Knox-Davies, 1985) and by the correlation of these enzyme levels with the extent of damage to the plant (Olsson, 1989; Wijesundra *et al*., 1989; Cleveland and Cotty, 1991; Baayen *et al*., 1997). Moreover, a sequential production of enzymes appears to be needed for effective cell wall degradation. Any change in the sequence will make the cell wall resistant to pathogen (Vidhyasekaran, 1997).

The occurrence of rot in infected tissue and the fact that *L. theobromae*~~*asiodiplodia sp.*~~ is capable of producing several cell wall hydrolyzing enzymes *in vitro* and in planta have implied macerating enzymes in pathogenesis.

2.7 Fruit response during fungal infection

Plants have to defend themselves against a variety of microbial organisms to ensure survival growth and development. Because of that, plants have evolved complex, integrated systems against potential pathogenic microorganisms. These mechanisms include preformed physical and chemical barriers as well as inducible defenses, such as strengthening of the cell wall and activation of several biochemical pathways for the synthesis of different compounds (Ramonell and Somerville, 2002; Thatcher *et al*., 2005).

Postharvest infection of fruits may induce a number of alterations in their physiological and biochemical processes or in the host tissue constituents as a result of host-pathogen interactions. Changes may include acceleration of ethylene evolution, stimulation of the respiratory enzymes, enhanced pectolytic activity, altered protein synthesis, or polyamine synthesis. Tissue changes may include increased cell-wall soluble pectin, and changed organic acid and sugar contents (Barkai-Golan, 2001). Focusing on the biochemistry of the plant defense system, it has been shown that plants respond to a pathogenic attack by the induction of phenolic compounds, polyphenol oxidase and peroxidase as part of the defense system (Pearce *et al*., 1998).

2.7.1 Phenolic compounds

Phenolic compounds, as parts of an active defense response, have long been implicated in disease resistance in many horticultural crops (Ndubizu, 1976). Phenolic compounds contribute to resistance through their antimicrobial properties, which elicit direct effects on the pathogen, or by affecting pathogenicity factors of pathogens. However, they may also enhance resistance by contributing to the healing of wounds via lignification of cell walls around clear zones. Evidence strongly suggests that esterification of phenols to cell wall material is a common aspect of the expression of resistance (Friend, 1981).

Antifungal properties of phenolic compounds are frequently found in young fruit that have these compounds in higher concentrations than in the ripe fruit. These compounds play an important role in the maintenance of latency in the unripe fruit. Moreover, in vitro assays have shown that the phenolic compounds, chlorogenic acid and ferulic acid, directly inhibited *Fusarium oxysporum* and *Sclerotinia*

sclerotiorium. The high concentration of chlorogenic acid, the principal phenol in the peach fruit epidermis and subtending cell layers, present in immature fruit and in fruit from highly-resistant genotypes may contribute to the brown rot-resistance of these tissues by interference with the production of factors involved in the degradation of cutin, rather than by direct toxicity to the pathogen (Bostock *et al*., 1999).

Phenolic compounds were found to increase following potato tuber inoculation or wounding alone in both susceptible and moderately resistant tubers, suggesting that phenolic biosynthesis was induced. Examination of extracts of infected tuber discs revealed the presence of a number of phenolic compounds. These results indicated that free phenolic acids may be induced by infection or wounding, and are then removed as they are converted into lignin or cross-linked to cell walls (Ray and Hammerschmidt, 1998).

Ray and Hammerschmidt (1998) found that inoculation of potato tubers with *Fusarium sambucinum*, the fungal pathogen causing potato dry rot, resulted in an increase in phenolic acids suggesting that phenolic synthesis was induced. Following such induction, free phenolic acids ~~are~~ were removed as they ~~are~~ were converted into lignin or are cross-linked to cell walls. Lignin production ~~is~~ was mediated by peroxidase, which ~~is~~ was strongly induced in the infected tuber in a number of isoforms. Furthermore, the phenolic acid and total peroxidase contents ~~are~~ were not higher in tubers of the resistant line than in more susceptible genotypes.

Accumulation of cell wall bound phenolics in fungi-inoculated was reported in many plant tissues, such as *Fusarium oxysporum* f. sp. dianthi-infected carnation stems. Bernards and Ellis (1991) reported that tomato cell cultures inoculated with *Verticillium albo-atrum* accumulated up to five-fold higher levels of wall-bound phenolics than were found in uninoculated cultures. The amounts of accumulation in resistant varieties normally was higher than in susceptible varieties (Cohen *et al*., 1990; Clark *et al*., 1994).

2.7.2 Polyphenol-oxidase (PPO)

PPOs are ubiquitous plant enzymes catalyzing the oxygen-dependent oxidation of mono- and o-diphenols to their corresponding o-quinones. These quinones are

reactive species that lead to the generation of polymeric dark pigments, which are responsible for the browning of fresh and processed fruits and vegetables.

The increase in PPO activity in infected or wounded tissues of many plants supports the assumption that PPO-mediated oxidative browning could be an important defense response of plants against infection or wounding. However, induction of PPO activity in an injured plant is not widespread, as PPO activities of a number of ~~plants~~ species did~~o~~ not increase after wounding (Constabel and Ryan, 1995; Gooding *et al*., 2001). PPO activities could be induced locally at infected or wounded sites of the tissues (Boss *et al.,* 1995; Ray and Hammerschmidt, 1998) or systemically perhaps to protect the plant against further attack (Thipyapong *et al.,* 1995) or both (Bashan and Henis, 1987; Constabel *et al*., 1995; Thipyapong *et al*., 1997). ~~–~~Hence, either constitutive, induced or both types of PPO activity may be involved in the putative protective defense strategy.

An increase in the amount of PPOs and PPO mRNAs was observed in wounded tissues of apple~~,~~ and potato (Boss *et al*., 1994; Thipyapong *et al*., 1995). Cleavage of an active PPO protein to a smaller and still active PPO form, resistant to further proteolysis and exhibiting a broad optimum pH, upon disruption of the infected cells is considered an alternative defense mechanism against pathogens (Mari and Guizzandi, 1998). ~~–~~Furthermore, stimulation of PPO activity by phytohormones associated with formation of new multiple forms, a consequence of the association of preformed low molecular weight multiple forms, may be another type of defense response (Saluja and Sachar, 1982). Whatever the mechanism is to control the level of PPO activity, disruption of membrane integrity, loss of latency, and transcriptional or posttranscriptional control during normal growth and development, PPO is ~~relatively~~ present at all developmental stages of plants and could even be more abundant after wounding or infection.

PPO is not the only enzyme contributing to resistance of plants to diseases. A study by Ray and Hammerschmidt (1998) on potato tuber supports involvement of constitutive PPO activity in cooperation with wound inducible peroxidase activity in a plant's defense resistance responses. PPO increase~~d~~s following inoculation with fungi, but to a lesser extent than peroxidase activity. The greatest ~~polyphenol oxidase~~PPO activity was found in tissue adjacent to the diseased zone, so it seemed to

be associated with fungal resistance and was highest in the more resistant potato varieties than in the susceptible ones. Furthermore, resistant tubers also browned more quickly than the susceptible ones following infection or wounding.

2.7.3 Peroxidase (POD)

Plant peroxidases are enzymes involved in the oxidation of compounds at the expense of H_2O_2. They play a key role in several aspects of plant physiology and development, such as lignification and suberization of cell walls, IAA oxidation, and post-harvest deterioration of fruits and vegetables. An increase in peroxidase activity generally occurs after wounding, incompatible pathogenic interactions and physiological stress (Campa, 1991). In several plants, infection by pathogenic fungi results in increased peroxidase activities. Accumulation of lignin and phenolic compounds has been correlated with disease resistance in a number of plant–pathogen interactions. POD activity was delayed or remained unchanged during the compatible interaction in susceptible plants. Ray and Hammerschmidt (1998) reported that peroxidase activity, which is very low in uninfected tuber, increased up to 500 fold after *Fusarium sambusinum* inoculated in potato tuber. Lignin content increased in tubers following inoculation, the highest concentrations being detected 40 h after inoculation. Boudjeko *et al*. (2005) found that 2 days after *Pythium myriotyrum* inoculation of cocoyam (*Xanthosoma sagittifolium* L. Schott), peroxidase activity of a resistant cultivar increased by 41%, while the increase in the sensitive and ~~mediate~~ moderately resistant cultivars were only 13.4 % and 7.5%, respectively.

ที่จัดรูปแบบ: การจัดเด็มแนวที่กระจายแบบไทย, ไม่ปรับช่องว่างระหว่างข้อความละดิน และข้อความเอเชีย

ที่จัดรูปแบบ: การจัดเด็มแนวที่กระจายแบบไทย

2.8 Control of Postharvest disease on longan

Longan fruit has ~~a high~~high sugar content in aril tissue which makes it easily rotted due to microorganisms, especially fungi. Many pre-harvest and post-harvest methods have been reported to control post-harvest loss in longan. In this area only methods related to this research are described.

2.8.1 Sulphur dioxide fumigation

The most common commercial means of preventing decay and browning is by using SO_2 due to its effectiveness and low cost. ~~The~~ SO_2 displays a wide range of

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useful effects in fruit. It can inhibit non-enzymatic browning resulting from the formation of mellanoidin pigments. As an antioxidant, it also can inhibit enzymatic browning~~;~~ involving oxidation of phenolic compounds, and it can be used as a disinfectant (Maurice *et al*., 2002). Sulfite compounds inhibit the growth of bacteria, yeast and fungi. Their efficiency depends on many factors but the most important factor is pH, with a lower pH resulting in greater efficiency. SO_2 and its derivatives (salts) react with water to be sulfurous acid. Types and the amount of ions depend on the pH of the solution. If the pH is greater than 7, SO_3^{-2} is the major form. If the pH is less than 4.5, HSO_3^{-1} is the major form. SO_2 will react with acetaldehyde of the microorganisms and can reduce the disulfide bonds of PPO in the plant cells as well. Moreover, it will react with some compounds and irritate the ~~microorganisms~~microorganisms' respiratory system. If the pH is less than 3, sulfuric acid is the major form. This form has the most inhibitory effect on the growth of microorganisms. It will penetrate into the microorganisms and destroy the cells (Boonyong, 2001). When the cell absorbs SO_2, it will interfere with membrane transport processes. Moreover, it also acts as a nucleophile cleaving the disulfite bond of proteins, reacting with coenzymes (NAD^+), cofactors, and prosthetic groups (flavin, thiamine, heme, folic acid, and pyridoxal). Consequently, a broad range of enzymes is inactivated, and structural proteins that contain disulfide bonds may be denatured. There is a rapid decrease in ATP content prior to the death of cell.

Rinpol (2005) reported that the shelf life of SO_2 fumigated longan fruit during storage at 10°C and 5°C were 3 and 6 weeks, respectively. Longan flesh pedicel turned brown after storage at 10°C for 4 weeks and turned yellow after storage at 5°C for 7 weeks.

2.8.2 Cold storage

Cold storage is the main method for reducing postharvest loss due to the delay of fruit physiological change and pathogen development. However, the possibility of lowering storage temperature is limited by the sensitivity of the fruit to chilling injury. This sensitivity varies among fruit species, or depends on the maturity stage of each cultivar (Barkai-Golan, 2001). Following chilling injury, the sensitivity of fruit to decay increases considerably.

ที่จัดรูปแบบ: ตัวยก

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Shelf-life and decay of longan fruit depend on storage temperature. Decay symptoms and fungi-covered peel and peduncle were observed in longan fruit, both non-inoculated and inoculated ~~fruit~~ with ~~caused~~ decay-causing fungi, during storage at 5°C for 22 days. A 20 h delay before storage at 5°C resulted in longan fruit decay within 18 days (Nachaiwieng, 1995). Consequently, many reports have shown that longan fruit decay and fungi-covered fruit were found within 22 days during storage at 5°C.

ที่จัดรูปแบบ: การจัดเต็มแนวที่กระจายแบบไทย, ไม่ปรับช่องว่างระหว่างข้อความละติน และข้อความเอเชีย

ที่จัดรูปแบบ: การจัดเต็มแนวที่กระจายแบบไทย

2.8.3 Coating

The function of coating materials on fruit is to cover or replace natural wax on its skin in order to reduce the transfer of gases including water vapor through its skin. Surface coating not only affects transpiration but also influences the internal atmospheric composition of the fruits. Moreover, coating substances such as chitosan have antifungal activity. An application of chitosan–coating (0.5, 1.0 and 2.0%) on longan kept at 2°C and 90% relative humidity reduced respiration rate and weight loss, delayed the increase in PPO activity and partially inhibited decay of fruit during storage (Jiang and Li, 2001). ~~Some research reported chitosan coating in longan with hexanal~~

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Srichart (2002) reported that longan fruit coated with 15% palm oil, 10% soybean oil, 2% chitosan, 5% sweet cassava flour,1% cooked flour rice, 5% Sta-fresh ~~and~~ or 1% townyaimom flour only reduced weight loss –compared with controls, with~~hereas~~ fungi ~~were~~ found on all parts~~iece~~ of the peel~~s~~ and peduncle upon PDA isolation.

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2.8.4 Natural volatile compounds

Only one ~~research~~ study to date has~~was~~ reported the effect of~~to~~ fumigation with ~~e~~ a natural volatile compound ~~in~~ of longan fruit. Rasrinaul (1996) found that acetaldehyde fumigation of longan fruit at~~,~~ 0.125% for 8 h ~~of~~ using cv. Daw~~,~~ was effective for control of postharvest decay. Its effectiveness depended on concentration, fumigation time and cultivar. Low concentrations could not control postharvest loss. Fumigation time had no effect when concentration was at a high level. ~~Moreover~~However, the effective concentration of acetaldehyde affected the

quality of the fruits ~~such as~~including darkening the color of the inner surface of the peel, yellowing and loss of the natural odor of the fresh fruit.

2.9 Plant natural volatile compounds

SO_2 fumigation is facing increasing consumer and regulatory resistance to its use due to the allergic reaction of some people to sulfite residues. At this time, commercially acceptable or safer alternative postharvest treatments are not available. Thus, finding an alternative method to replace SO_2 fumigation is necessary. There is an increasing demand by consumers for consistently safe, healthy food with good flavor and quality. Therefore, using natural products with fungistatic properties may be an attractive alternative to prevent decay.

Plants and plant products emit a wide range of volatile compounds, some of which are important flavor quality factors in fruits, vegetables, spices and herbs (Kays, 1991). Wilson *et al*. (1987) reported that a number of natural volatile compounds including "wound volatiles" have anti-fungal effects. Among these antifungal compounds are aldehydes, ketones, alcohols, acid, amines and several additional chemical classes. In addition to their antifungal effect, the volatile compounds can be metabolized by fruit and may leave little to no residue

Several volatile compounds are produced in virtually all green plant tissues in response to mechanical or biological injury (Hatanaka, 1993). These wound volatiles, usually six- and nine–carbon aldehydes or alcohols, are formed via the lipoxygenase (LOX) hydroperoxide lyase enzymatic pathway (Hildebrand, 1989), which are activated immediately following wounding. The antimicrobial activity of two major wound volatiles, hexanal and *trans*-2-hexenal, was reported by Gardini *et al*. (1997) and Kubo and Fujita (2001). The effectiveness of hexanal as a metabolizable fungicide and its enhancement of aroma production by interconversion to other aroma volatiles in minimally-processed apples were observed by Song *et al*. (1996, 1998). Moreover, as shown by Lanciotti *et al*. (1999), the addition of hexanal at levels not exceeding 100 ppm in the storage atmosphere of fresh ~~sliced~~ apple slices had a positive effect on their quality. In fact, it improved shelf life by reducing the growth rate of naturally-occurring microbial populations during storage at 4 and 15°C. When added to a modified atmosphere (70% N_2 and 30% CO_2), hexanal was also very

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effective in slowing the browning reaction at 15°C, maintaining an acceptable color for 16 days from packaging. Change in hue angle values was not observed in the samples stored at 4°C in the same period. This latter effect was attributed to hexanal conversion to hexanol because the aliphatic alcohols are regarded as inhibitors of PPO (Valero *et al*., 1990). Another possible target of hexanal could be phenylalanine ammonia-lyase, a key enzyme for polyphenol biosynthesis, whose production can be activated by tissue injuries and ethylene (Lanciotti *et al*., 1999).

Trans-2-hexenal is strongly antifungal in nature and is metabolized as well, with activity against *Botrytis cinerea* (Hamilton-Kemp *et al*., 1992; Archbold *et al*., 1997a; Fallik *et al*., 1998). Archbold *et al*. (1999) reported that *trans*-2-hexenal was an efficient fumigant in controlling mold on seedless table grapes. The inclusion of hexanal in combination with *trans*-2-hexenal in the atmosphere of fresh apple slices yielded a significant extension of shelf life, even when a spoilage yeast such as *Pichia subpelliculosa* was inoculated and an abusive storage temperature was used (Corbo *et al*., 2000).

Moreover, several plant natural volatile have been identified for their antifungal activity *in vitro*. Archbold *et al*. (1997b) reported hexanal, 1-hexanol, *trans*-2-hexen-1-ol, *cis*6-nonenal, *trans*-3-nonen-2-one, methyl salicylate and methyl benzoate had potential as postharvest fumigants for control of strawberry diseases. Strawberry, blackberry, and grape metabolized *trans*-2-hexenal with reduction of aldehyde to alcohol. Strawberry had a level of metabolism higher than blackberry, and grape, which led to a variation in species sensitivity to the volatile compound where blackberry and grape showed no phytotoxicity effect in contrast to strawberry. Vaughn *et al*. (1993) reported that benzaldehyde at 0.04 ~~µL~~ µl ~~L~~l^{-1} completely inhibited ~~—~~*Alternaria~~-~~ alternata*, *B. cinerea* and *Colletotrichum~~-~~ gloeosporioides*, while 1-hexanol, *trans-2*-hexenal and 2-nonanone inhibited the three fungal species at 0.1 ~~µL~~ µl ~~L~~l^{-1}. Utama *et al*. (2002) found acetaldehyde, benzaldehyde, cinnamaldehyde, ethanol, benzyl alcohol, nerolidol and 2-nonanone had antifungal activity against the fruit and vegetable pathogens, *Penicillium~~-~~ digitatum*, *Rhizopus~~-~~ stolonifer*, *Colletotrichum~~-~~ musae* and *Erwinia~~-~~ ~~C~~caratovora*, during *in vitro* trials. Benzaldehyde has been used in the laboratory to fumigate peaches and to protect them

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against Rhizopus rot, and it totally inhibited spore germination of *B. cinerea* at 25 µg ~~l~~l^{-1} and germination of *Monilinia~~,~~ fructicola* at 125 µg ~~l~~l^{-1} (Wilson *et al*., 1987).

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2.10 Factors effecting efficacy of natural volatile compounds

The effectiveness of volatile compounds in inhibiting microbial growth is varied. The C_6 and C_9 aldehyde and alcohol products were more effective than the ester products in inhibiting *B. cinerea* growth in *in vitro* bioassays (Hamilton-Kemp *et al*., 1992). An α,β-unsaturated bond adjacent to the carbonyl moiety may enhance antifungal activity (Anderson *et al*., 1994). The aldehydes (acetaldehyde, benzaldehyde, and cinnamaldehyde) were found to be stronger inhibitors of growth than other tested compounds and caused germicidal effects both on spores and mycelia of fungi (*C. musae~~,~~* and *P. digitatum*) and on bacterial cells (*E. carotovora* and *Pseudomonas~~.~~ aeruginosa*). The ketones (2-nonanone and β-ionone) and the ester (ethyl formate) tended to be effective only against specific decay microorganisms. 2-Nonanone was germistatic against *P. digitatum* and *C. musae*, and β-ionone was germistatic against *C. musae* only. Ethyl formate was germicidal against *R. stolonifer*, *C. musae*, *E. carotovora* and *P. aeruginosa*, but did not completely inhibit the growth of *P. digitatum* (Utama *et al*., 2002). The germicidal effects of the tested aldehydes were through the destruction of spores and shrinkage of mycelia of decay fungi, so that complete inhibition~~ors~~ of both spore germination and mycelial growth was achieved. The strong inhibition of the mycelia by the aldehyde seems similar to the finding that acetaldehyde caused leakage of electrolytes from fungal mycelia (Avisar *et al*., 1990).

The water solubility and boiling point of volatile compounds may have an effect on their inhibition of fungal growth *in vitro*. For example, 2-nonanone and β-ionone are non-water-soluble volatiles and have high boiling points, 196 and 129°C, respectively. These factors may limit the amount of vapor that comes into contact with the microorganisms, so that the inhibition remains incomplete. Ethyl formate, a slightly water-soluble volatile, has also been found ineffective in inhibiting the growth of *P. digitatum*-inoculated oranges. The amount of vaporized volatile in the headspace and the amount of its vapor that is then absorbed by the medium are important factors determining the effectiveness of a volatile against decay

microorganisms. In an agar plate bioassay system, the distribution of volatile compounds could be dependent on the water solubility of the volatiles. Volatiles were readily incorporated into the agar medium: approximately 70% were absorbed and <0.5% of the volatiles remained in the headspace after 1 h at 25°C. Benzaldehyde and cinnamaldehyde, however, are only very slightly soluble in water (Fenaroli, 1995) and were absorbed by the medium to a concentration of only about 20 and 30%, respectively, with a low level in the headspace. However, despite the lower amount found in the agar medium compared with the water-soluble volatiles, benzaldehyde and cinnamaldehyde were able to inhibit the growth of microorganisms completely.

Antifungal activity of some volatile compounds such as hexanal is apparently related to vapor pressure rather than on their whole concentration in the system. The vapor pressure of a molecule, at a given concentration, depends on temperature. A temperature increase enhanced the antifungal activity of hexanal due to its effect on vapor pressure. But a high water vapor pressure did not affect the vapor pressure of hexanal, due to its very poor water solubility (Gardini *et al*., 1997).

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Duration of volatile exposure is another factor to be considered. A continuous exposure of fruit to volatiles may be required for some of the compounds to have an inhibitory effect, while others may be effective with exposures of short duration. A single day of treatment with *trans*-2-hexenal effectively inhibited *B. cinerea* for up to 13 days (Archbold *et al*., 1997b). In contrast, continuous exposure to methyl salicylate was necessary to suppress *B. cinerea* development. Furthermore, the *trans*-2-hexenal hastened quality deterioration while methyl salicylate had no effect on fruit quality.

However, *in vitro* study alone cannot predict the effectiveness of volatiles on horticultural produce, because their effectiveness also depends on their interaction with, metabolism by, and phytotoxicity to the produce exposed to them. Thus, experiments need to be done under *in vivo* conditions, especially when the minimum inhibitory concentration in produce is relatively high. The potential use of volatile fungicides to control postharvest diseases requires a detailed examination of their biological activity and interaction with fruit tissue, and the development of a method which inhibits the growth of pathogens at non-phytotoxic concentrations. Furthermore, differences in response may exist, depending on the species being

tested. From their antifungal effect alone, a number of volatile compounds have exhibited promise as postharvest fumigants to control mold. However, many compounds hastened postharvest deterioration of fruit quality. It may be possible to retain the antifungal effect and reduce the adverse impact on fruit quality by reducing the length of the treatment period, lowering the vapor phrase concentration, or by using analogues of the volatile compounds. The specific strategy will need to be defined for each compound, as shown by the difference in effectiveness of *trans*-2-hexenal and methyl salicylate with varying durations of exposure (Archbold *et al*., 1997b). However, the use of these volatile compounds as antimicrobial agents can be a valuable field of investigation: toxicity to mammals is quite low, and the degree of volatility allows their use for fumigation in cold storage or for active packaging

2.11 Hexanal properties

Hexanal has synonyms including caproic aldehyde, capronaldehyde, 1-hexanal, hexanaldehyde, and hexylaldehyde. Its molecular formula is $C_6H_{12}O$ and molecular weight is 100.16. Hexanal is a clear liquid with a fruity taste. Its density is 0.814 (g cm^{-3}), melting point is less than -20°C, and boiling point is 119-124°C. It is classified as a generally recognized as safe (GRAS) compound, and is used for fruit flavoring for apple, apricot, banana, bilberry, black currant, blueberry, cranberry, grape, guava, kiwi, loganberry, mango, muskmelon, passion fruit, peach, pear, pineapple, plum, quince, raspberry, strawberry and citrus flavors. Its use level is between 0.005 to 4 ppm when consumed. It occurs naturally in apple, avocado, black currants, blueberry, coconut, cucumber, honey, passion fruit, peach, raspberry, strawberry, and tomato among many. It is soluble in alcohol, oil and very slightly soluble in water. It has an ORL-RAT LD50 of 4890 mg kg^{-1}, ORL-MUS LD50 of 8292 mg kg^{-1} and ORL-MAM LD50 of 3700 mg kg^{-}.

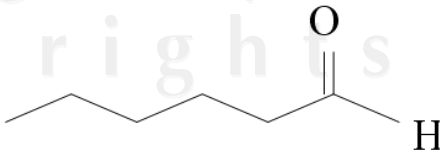

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Figure 2.2 Molecular structure of hexanal

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2.12 Using hexanal to control fruit decay *in vitro* and *in vivo*

In recent years the interest in using plant volatiles to prevent fungal and bacterial growth has ~~more~~ increased. Hexanal has been reported to have antifungal activity against many postharvest pathogens and has potential to control fruit decay in some crops.

Song *et al.* (1998) found that hexanal fumigation at 450 ~~µL~~ µl ~~L~~l^{-1} for 48 h completely inhibited fungal growth of *P. expansum* Link and *B. cinerea* Pers. on PDA media including retarding~~ed~~ decay lesion development on 'Golden Delicious' and on 'Jonagold' apple slices. Hexanal treatment stimulated aroma volatile production of hexanol and hexyl acetate by apple slices of both cultivars after 20-30 h of treatment. Neri *et al.* (2006) reported a fungistatic concentration of hexanal was 196.8 ~~µL~~ µl ~~L~~l^{-1} for conidial germination and 98.4 ~~µL~~ µl ~~L~~l^{-1} for mycelium growth of *P. expansum.* Almenar (2007) evaluated the fungistatic and fungicidal effects of hexanal *in vitro* against *C. acutatum*, *A. alternata* and *B. cinerea*, the three main causes of postharvest diseases in berries, for 7 days at 23 °C. Results showed that its effectiveness was greater against *C. acutatum* than *A. alternata* and *B. cinerea*. Concentrations of 1.1, 2.3 and 1.3 µ~~L~~l ~~L~~l^{-1} ~~µL hexanal/L~~ air, ~~respectively,~~ were necessary to prevent *C. acutatum*, *A. alternata* and *B. cinerea* growth, respectively. Lower concentrations reduced fungal growth depending on the included amount and type of fungus. ~~Moreover, h~~Hexanal was inserted into β-cyclodextrins (β-CD) to develop a controlled release mechanism due to its highly volatile nature, but~~. Though~~ the same amount of hexanal released from β-cyclodextrin had a lower antifungal effect on *C. acutatum*. Inclusion complexes (ICs) such as *β*-cyclodextrin-hexanal can be used to reduce or avoid post-harvest berry diseases because of their capacity to

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provide an antifungal volatile during storage, distribution, and consumer purchasing (Almenar *et al*., 2007).

Utto *et al.* (2008) reported continuous hexanal exposure of tomato effectively suppressed grey mould with the minimum inhibitory concentration (MIC) being 40–70 ~~µl~~ µl ~~l~~l^{-1}. During continuous exposure at the MIC the fruit respiration rate was increased 50% and reddening was slowed during storage for 7 days at 20±1 ˚C and 99% RH.

ที่จัดรูปแบบ: ตัวยก

ที่จัดรูปแบบ: การจัดเต็มแนวที่กระจายแบบไทย, แท็บหยุด: 4.68", ซ้าย

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2.13 Mode of action

The mode of action of these volatile compounds on fungi has not been established. *Trans*-2-hexenal and related LOX products are all lipophilic and may alter membrane permeability of fungal spores, as well as fungal metabolism (French, 1985; Fries, 1973). Avisar *et al*. (1990) reported that the germicidal effects of the tested aldehydes were through the destruction of the spores and shrinkage of mycelia of decay fungi, so that complete inhibition of both spore germination and mycelia growth was achieved.

Many reports indicate the cell membrane as the primary target of bioactive aroma compounds. Membrane disruption by terpenes has been observed in bacteria and fungi (Cox *et al*., 2000; Helander *et al*., 1997). –The hydrophobicity of the molecules affects their solubility in the cell plasma membrane and their bioactivity depends firstly on their partition coefficient (Caccioni *et al*., 1997; Lambert *et al*., 2001). The lipophilic character of cyclic monoterpenes favors their partition from an aqueous phase into cell membranes, with a consequent expansion and increased fluidity, leading to an inhibition of the membrane-embedded enzymes (Cox *et al*., 2000). Lipophilicity was used to describe the activity against *Listeria monocytogenes* of cinnamic acid and benzaldehydes (Ramos-Nino *et al*., 1996). Since the bioactivity of many aroma compounds is dependent on their partitioning into the plasma membrane, a key role in their toxicity may be attributed to the vapor pressure that can be considered an indirect measure of their hydrophobicity. Thus, factors able to increase the vapor pressure of these substances can enhance their antimicrobial activity increasing their solubility in cell membranes. Lanciotti *et al.*, (1999) proved that antimicrobial activity of hexanal, *trans*-2-hexenal and hexyl

acetate was dependent on their vapor pressure and, consequently, positively affected by temperature rise. A temperature rise increases the tendency of a molecule to pass into the vapor phase and, consequently, improve its antimicrobial effects.

Myung ~~*et al.*~~ (2005) found an effect of exposure of *trans*-2 hexanal at 5.4 and 85.6 µmol ~~L~~l^{-1} on protein expression in *B. cin*erea, ~~with~~ and 95% of the *trans*-2-hexanal loosely bound to fungal tissue interacting with protein from the surface ~~(wash protein)~~ of the fungal tissue, while 5% was interacting with protein in internal tissue (cell wall, membrane, and cytosol). Proteins recovered ~~in the wash fraction~~from the fungal surface may be those secreted from mycelia of *B. cinerea*, as the fungus is known to secrete cuticle and cell wall-degrading enzymes (Staples and Mayer, 1995). Interaction of C6 aldehydes with the secreted proteins could modify them and thus affect the growth and pathogenesis of *B. cinerea* and other fungi. Protein modification at sufficient levels may cause loss of pathogenicity. Alberti-Segui *et al*. (2004) showed that the absence of glycosidase activity, a secreted protein from yeast, reduced adherence to target cells and delayed colonization. Fallik *et al*. (1998) found malformed and ruptured *B. cinerea* hyphae after *trans*-2-hexenal exposure. Arroyo *et al.* (2007) studied the effects of *trans-2*-hexenal on conidial cells of *C. acutatum* by transmission electron microscopy. This volatile compound altered the structures of the cell wall and plasma membrane, causing disorganization and lysis of organelles and, eventually, cell death.

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